SHORT COMMUNICATION

Assignment of the Gene Encoding the 5-HT_{1E} Serotonin Receptor (S31) (Locus HTR1E) to Human Chromosome 6q14–q15

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The human gene for the 5-HT_{1E} serotonin receptor was recently cloned, but no chromosomal assignment has yet been given to this gene (locus HTR1E). In this work, we demonstrate by two independent polymerase chain reactions on a panel of human–hamster somatic cell hybrid genomic DNA that the 5-HT_{1E} serotonin receptor gene is localized on human chromosome 6. Furthermore, by means of *in situ* hybridization to human metaphase chromosomes, using the cloned 5-HT_{1E} receptor gene (phage clone λ-S31; Levy *et al.*, *FEBS Lett.* 296:201–206, 1992) as a probe, we demonstrate that this gene is localized to the q14–q15 region on chromosome 6. Screening of genomic DNA from 15 unrelated Caucasian individuals, using as a probe the open reading frame of the cloned 5-HT_{1E} receptor gene, did not reveal any restriction fragment length polymorphisms with the enzymes *Bam*HI, *Ban*II, *Bgl*II, *Eco*RI, *Hinc*II, *Hin*dIII, *Hin*fI, *Msp*I, *Pst*I, and *Pvu*II. Since the 5-HT_{1E} receptor is found mainly in the cerebral cortex and abnormal function of the serotonergic system has been implicated in a variety of neurologic and psychiatric diseases, the precise chromosomal assignment of the 5-HT_{1E} receptor gene is the crucial first step toward the evaluation of this locus as a candidate for mutations in such syndromes.

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter and vasoactive substance with a variety of biological effects, both in the central nervous system and in the periphery. The many different physiological functions regulated by serotonin, e.g., sleep, mood, intestinal motility, and blood pressure, point to important roles for serotonin in the pathogenesis of diverse pathological conditions such as anxiety, depression, migraine, nausea, vasospasm, and hypertension (2, 18). All known biological effects of serotonin are mediated via receptors in the cell membrane that fall into two classes, those coupled to signal-transducing G proteins (receptors belonging to the 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 families) and ligand-gated ion channels (5-HT_3 receptors) (14, 21, 24, 28).

We recently identified by molecular cloning a novel human gene (first referred to as S31) encoding a serotonin receptor that we and others have later classified as the human 5-HT_{1E} receptor (4, 10, 15, 27). This receptor mediates inhibition of adenylyl cyclase and is found primarily in human cerebral cortex (9, 10).

To determine the chromosomal localization of the 5-HT_{1E} receptor gene, a panel of 25 human–hamster somatic cell hybrids was analyzed for the presence or absence of two independent PCR amplification products. Genomic DNAs (50 ng) from human–hamster somatic cell hybrids (BIOSMAP Somatic Cell Hybrid PCRable DNAs, BIOS, New Haven, CT) were used as template. PCR was performed according to standard protocols (22). To minimize nonspecific amplification, a "touch-down" PCR protocol was used, with annealing temperature lowered by 1°C for each cycle, starting at 70°C and ending up with 20 cycles at 55°C, a total of 35 cycles, followed by 10 min at 72°C. Each cycle consisted of 45 s at 94°C, 45 s at annealing temperature, and 90 s at 72°C. PCR products were analyzed both by acrylamide gel electrophoresis and by Southern blot analysis (22). In both PCRs, four of the somatic cell hybrid DNAs, named SM 756, SM 860, SM 904, and SM 909, as well as human genomic DNA and pBS-S31.2 plasmid DNA (10), but not hamster genomic DNA, yielded PCR products of the expected length (584 bp for primers A + B, 390 bp for primers C + D) (Figs. 1A and 1B). To ensure that the amplified DNA was the 5-HT_{1E} receptor and not a homologous serotonin receptor, the correct identities of the amplified PCR products were confirmed by two independent approaches. First, oligonucleotide probes (E and F) (Fig. 1A) were designed to recognize unique areas of the 5-HT_{1E} receptor sequence within the expected PCR products, showing essentially no homology to other cloned serotonin receptors. When hybridized to a Southern blot of agarose-fractionated, PCR-amplified DNA from

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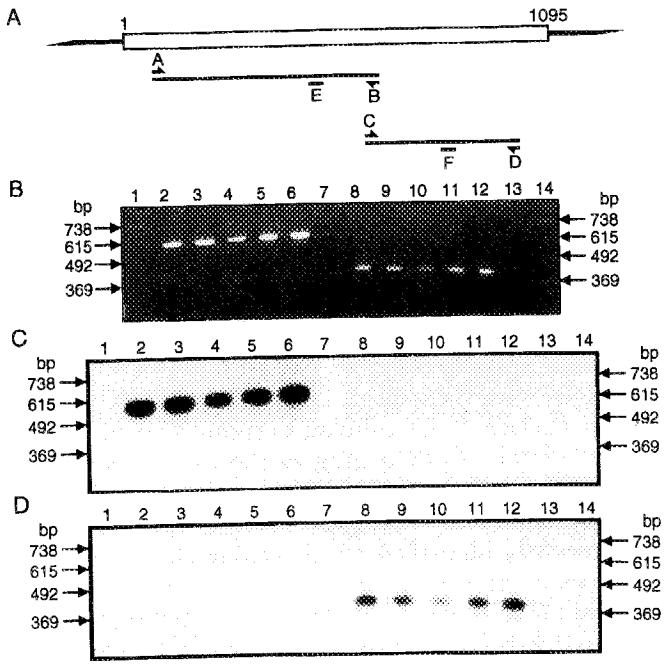

FIG. 1. (**A**) Schematic drawing of the 5-HT_{1E} receptor gene with the open reading frame indicated, illustrating the location of the two PCR products and the probes used to confirm their identity. (**B–D**) Agarose gel electrophoresis and Southern blot analysis of the PCR products obtained from genomic DNA (50 ng) of human (lanes **2** and **8**), hybrid cell lines SM 756, SM 860, SM 904, and SM 909 (lanes **3–6** and **9–12**), and hamster (lanes **7** and **13**), using as primers oligonucleotides A and B (lanes **2–7**) or C and D (lanes **8–13**). Lanes **1** and **14**, 123-bp DNA ladder (Bethesda Research Laboratories, Bethesda, MD). (**B**) Agarose gel stained with ethidium bromide. (**C, D**) Southern blot of the agarose gel shown in **B** hybridized with oligonucleotides E (**C**) or F (**D**). The hybrid cell lines not yielding PCR products were SM 212, SM 324, SM 423, SM 507, SM 683, SM 734, SM 750, SM 803, SM 811, SM 854, SM 862, SM 867, SM 937, SM 940, SM 967, SM 968, SM 983, SM 1006, SM 1049, SM 1079, and SM 1099. The oligonucleotides were (5′ → 3′; numbers refer to the published sequence of the human 5-HT_{1E} receptor (S31) (10)): A, TTTGCATGACTCTGGTGGTC (74 → 93); B, ACTTGATCCCCTTTTCTGG (657 → 639); C, GCCAAGAGCCTTTACCAG (625 → 642); D, CAGAGAATTCACATAACCG (1014 → 996); E, AGGGGGAGGGCTTAGGCGGCGGTGGCT (510 → 484); F, TCTGCTGACGTTCTCCTGGGTGATCTA (841 → 815).

the positive somatic cell hybrids as well as human and hamster genomic DNA (Fig. 1B), probe E but not probe F recognized the PCR product from primers A + B, and probe F but not probe E recognized the PCR product from primers C + D, as expected from the design of the PCR products (Figs. 1C and 1D). Second, PCR products obtained with each primer set were subcloned into the pCR II vector (Invitrogen, San Diego, CA) and sequenced from both ends by the dideoxy chain termination method (23). The sequences obtained matched exactly the sequence of the human 5-HT_{1E} receptor, confirming unequivocally that the PCR products represented the 5-HT_{1E} receptor gene and not a homologous receptor gene.

Discordancy analysis of the results from the 25 somatic cell hybrids (Table 1) revealed a 100% concordancy for chromosome 6, whereas all other chromosomes were excluded (discordancy 16% or higher).

The chromosomal mapping position of the phage clone λ-S31 (10), containing approx. 15 kb of the human 5-HT_{1E} receptor gene, was also determined by fluorescence *in situ* hybridization (FISH). Metaphase chromosomes were prepared following standard procedures (26). Hybridization was performed as described earlier (19, 20), using 80 ng probe (ca. 15 kb *Sal*I–*Sal*I insert of clone λ-S31) labeled with biotin–11-dUTP via nick-translation. Recent improvements in FISH protocols and the introduction of highly sensitive detection devices such as a cooled CCD camera (PM512, Photometrics, Tucson, AZ) used in the described experiments allow for the visualization of target sequences even without immunological signal amplification steps. To assign the chromosomal mapping position with respect to cytogenetically defined bands, chromosomes were counterstained with DAPI, which revealed a G-banding

TABLE 1

Discordancy Analysis of the Results of PCR Amplification of the Human 5-HT_{1E} Receptor Gene in Human–Hamster Somatic Cell Hybrids

Human chromosome	Concordant		Discordant		Discordancy (%)
	+/+	−/−	+/−	−/+	
1	0	18	4	3	28
2	0	20	4	1	20
3	1	18	3	3	24
4	0	19	4	2	24
5	4	3	0	18	72
6	4	21	0	0	0
7	1	20	3	1	16
8	1	17	3	4	28
9	0	18	4	3	28
10	1	19	3	2	20
11	0	18	4	3	28
12	2	19	2	2	16
13	1	16	3	5	32
14	2	16	2	5	28
15	0	17	4	4	32
16	1	19	3	2	20
17	0	19	4	2	24
18	0	17	4	4	32
19	2	16	2	5	28
20	1	19	3	2	20
21	3	17	1	4	20
22	0	17	4	4	32
X	1	19	3	2	20
Y	2	19	2	2	16

Note. Two independent polymerase chain reactions gave identical results. The presence of a PCR product (584 or 390 bp) is correlated with the presence or absence of each human chromosome in the 25 cell hybrids tested. Concordance indicates the presence of both PCR products and the chromosome (+/+) or the absence of both (−/−). Discordance indicates the presence of the PCR products in the absence of the chromosome (+/−) or the absence of the PCR products in the presence of the chromosome (−/+). The percentage discordancy was calculated for each chromosome by dividing the number of hybrid cell lines in which the PCR products did not segregate with the chromosome by the total number of hybrid cell lines scored.

pattern. A representative metaphase after hybridization with the biotinylated probe is shown in Fig. 2. The signal is observed on chromosome 6q14–q15. A total of 15 randomly selected metaphases were evaluated: 13 revealed hybridization signals on both chromatids of the homologous chromosomes. Two metaphases showed signals on only one chromatid or only one homolog. Signals at other mapping positions were not observed.

To examine whether a probe from the 5-HT_{1E} receptor gene (a 1160-bp *Acc*I–*Bsp*HI fragment of the plasmid pBS-S31.2 (10), containing the entire open reading frame) could be used to detect polymorphisms, useful in, e.g., linkage studies, we screened genomic DNA from 15 unrelated Caucasian individuals, using methods described earlier (12). However, no RFLPs were detected among the 15 individual genomic DNA preparations tested using the enzymes *Bam*HI, *Ban*II, *Bgl*II, *Eco*RI, *Hin*cII, *Hin*dIII, *Hin*fI, *Msp*I, *Pst*I, and *Pvu*II (results not shown).

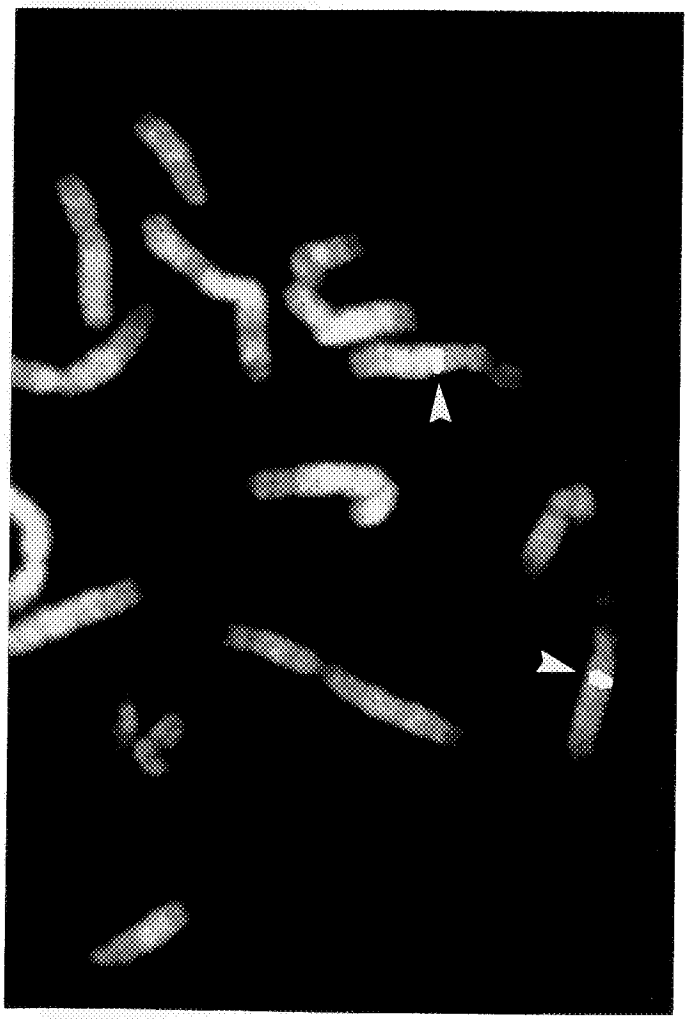

FIG. 2. Chromosomal localization of the phage clone (λ-S31) containing the 5-HT_{1E} serotonin receptor gene as determined by fluorescence *in situ* hybridization. Human metaphase chromosomes were prepared and hybridized to biotin–11-dUTP-labeled λ-S31 DNA, followed by washing, detection with avidin-conjugated fluorescein isothiocyanate, and counterstaining with DAPI as described previously (19, 20). The signals observed on chromosome 6q14–q15 are indicated by arrowheads.

TABLE 2

Chromosomal Localization of Human Genes for G-Protein-Coupled Serotonin Receptors

Receptor	Locus	Chromosomal localization	References
5-HT_{1A}	HTR1A	5cen–q11	(3, 8)
5-HT_{1B}/5-$HT_{1D\beta}$	HTR1B	6q13	(1, 7)
5-HT_{1C}	HTR1C	Xq24	(16)
5-$HT_{1D\alpha}$	HTR1D	1p36.3–p34.3	(13)
5-HT_{1E}	HTR1E	6q14–q15	This report
5-HT_{2}	HTR2	13q14–q21	(6, 25)
5-HT_{5A}	HTR5A	7q36	(14)
5-HT_{5B}	HTR5B	2q11–q13	(14)
5-HT_{1D} pseudogene	HTR1DP1	12	(17)

The demonstration that the human 5-HT_{1E} serotonin receptor gene is localized on chromosome 6q14–q15 places this gene in close proximity to another serotonin receptor gene, the 5-$HT_{1D\beta}$ receptor (7, 11) (locus HTR1B), which was recently assigned to chromosome 6q13 (1, 7). Both these genes contain intronless open reading frames and encode closely related serotonin receptors. The exact distance between these two closely related genes is not known, and speculations as to their possible common origin and evolution through gene duplication should await better knowledge of this chromosomal region. The localization of another G-protein-coupled receptor gene, encoding a cannabinoid receptor (locus CNR), to this region (6q14–q15) is also of interest (5). Table 2 lists the chromosomal localization of those human G-protein-coupled serotonin receptors for which such information is available. The eight different genes listed are localized on seven different chromosomes. In addition, a pseudogene for the 5-$HT_{1D\alpha}$ receptor is localized on yet another chromosome (Table 2). Hence, as yet, no specific pattern that could give clues to the evolution of this large gene family is apparent.

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